Brain Research Bulletin, Vol. 14, pp. 169–177, 1985. © Ankho International Inc. Printed in the U.S.A. 0361-9230/85 $3.00 + .00

Muscarinic Cholinergic Receptors in Area Postrema and Brainstem Areas Regulating Emesis

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Received 15 May 1984

PEDIGO, N. W., JR. AND K. R. BRIZZEE. *Muscarinic cholinergic receptors in area postrema and brainstem areas regulating emesis* BRAIN RES BULL **14**(2)169–177, 1985.—Central cholinergic pathways modulate both the perception of excessive motion stimuli and the expression of motion sickness symptoms, such as nausea and vomiting. Specific brainstem areas which mediate motion-induced emesis include the area postrema (AP), vagal nuclear complex (VNC), reticular formation (RF) at the site of the vomiting center, and the vestibular complex (VC). In this report, histological studies indicated the cellular organization of brainstem structures mediating emesis was similar in bovine and squirrel monkey brain. The objective of this study was to characterize biochemical and pharmacological properties of muscarinic cholinergic receptors assayed by ^{3}H-QNB binding in these regions of bovine brainstem. Scatchard analyses of specific ^{3}H-QNB binding showed an uneven distribution of muscarinic receptors, with high densities of sites in VNC and AP, intermediate levels in RF and lowest receptor concentrations in VC. Dissociation constants for ^{3}H-QNB, measured in saturation and kinetic experiments, were similar in all brainstem regions. The pharmacological potency of cholinergic agonists and antagonists was the same as reported for muscarinic receptors labeled in other brain areas or peripheral organs. Several drugs which potently inhibited ^{3}H-QNB binding in bovine brainstem also exhibited antiemetic activity in a squirrel monkey model of motion-induced emesis. The antimotion sickness effects of these drugs may be due, in part, to their antagonism of muscarinic receptors in brainstem areas regulating emesis.

Antiemetics Area postrema Motion sickness Muscarinic cholinergic receptors ^{3}H-QNB binding Vomiting center

NAUSEA and vomiting in humans may be precipitated by excessive motion stimuli, functional stimuli of visceral origin, or chemical stimuli, including various drugs. The brainstem reticular formation, especially in the region of the vomiting center, represents the common terminus of several afferent pathways which modulate emesis [3,4]. Chemical stimuli in blood or cerebrospinal fluid activate neurons in area postrema (AP), the so-called chemoreceptor trigger zone [2], which then stimulate the lateral reticular formation at the site of the vomiting center. Similarly, visceral afferents, primarily of vagal origin, act via the nucleus tractus solitarius (NTS) to stimulate the vomiting center [3]. Motion stimuli, sensed by hair cells of the inner ear, are transmitted to the brainstem vestibular complex (VC) and also can activate the vomiting center to produce emesis [18]. Another brainstem region regulating emesis is the dorsal motor nucleus of the vagus (DMV), which may modulate motor components of vomiting.

Motion sickness characteristically produces nausea, vomiting and retching, as well as anorexia, drowsiness, pallor, cold sweat and malaise in humans [8]. Such symptoms can have a debilitating or incapacitating effect on individuals, including sailors, pilots or astronauts subjected to unconventional motion stimuli. Space sickness, which is generally considered to be a form of motion sickness, has become a particular problem in recent Space Shuttle flights [27] and is the focus of considerable research by NASA's Biomedical Research Program [28]. One of the most effective treatments for motion or space sickness is the muscarinic antagonist scopolamine, but other drugs (promethazine, ephedrine, prochlorperazine, diphenhydramine, thiethylperazine) are also good antiemetics [27,29]. Studies of motion sickness in man reveal extreme intersubject variability in the therapeutic and adverse responses to antimotion sickness drugs.

The efficacy of scopolamine in preventing motion-induced emesis and other symptoms of motion sickness implies an increased activity of cholinergic neurons in response to unusual motion stimuli. The crucial role of central cholinergic pathways in the neurophysiology of motion sickness, in general, and space sickness, in particular, has been reviewed recently [16]. Hair cells in the vestibular apparatus of the inner ear, which respond to motion stimuli, are apparently excited by cholinergic neurons [11]. Acetylcholine also acts as a modulatory afferent neurotransmitter in brainstem vestibular nuclei [13] and may be involved in other brainstem areas which mediate the expression of motion sickness symptoms. Cholinergic systems in cortex, midbrain ascend-

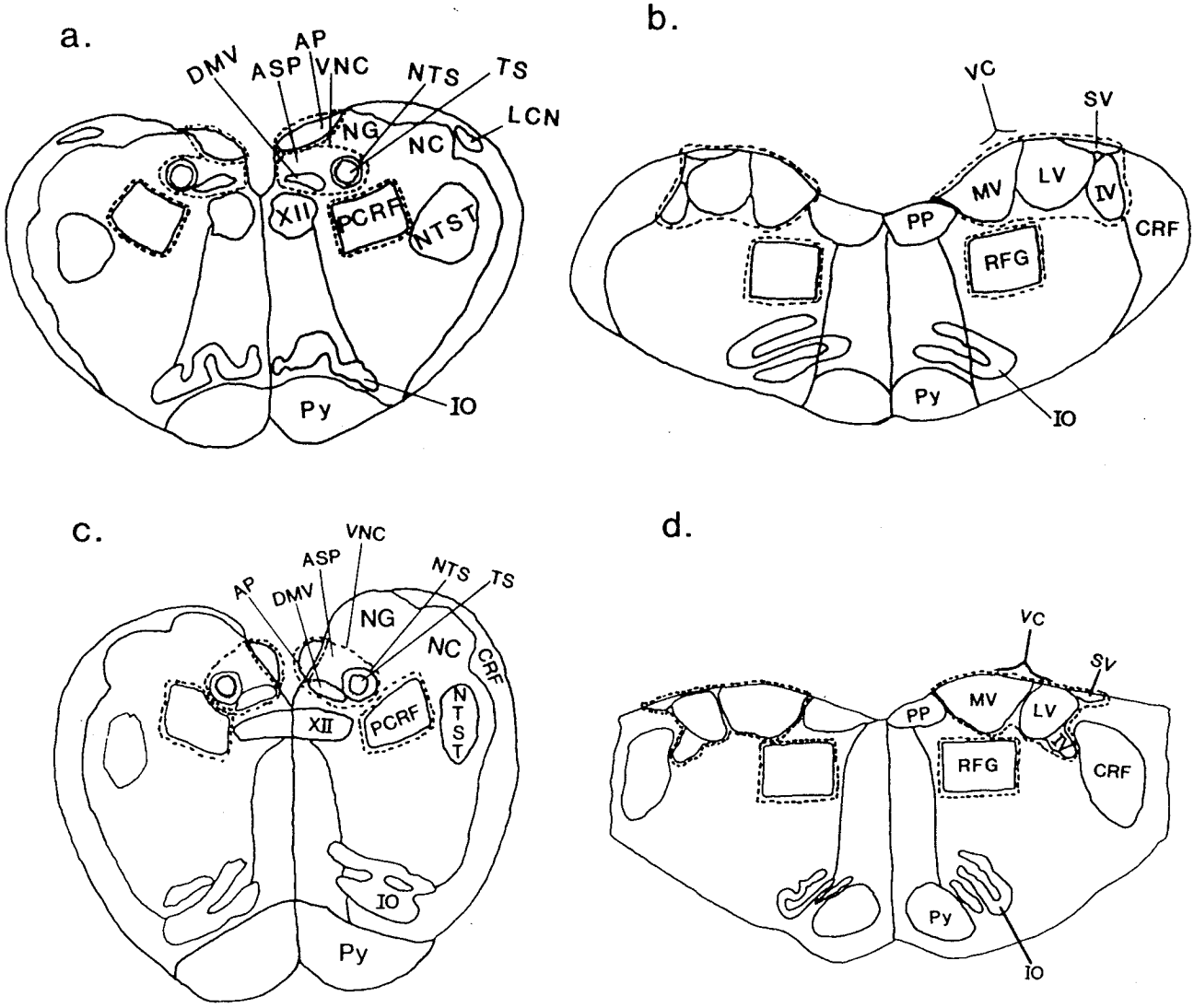


FIG. 1. Camera lucida outline drawings illustrating the dissection scheme employed for obtaining tissue samples from various structures of bovine (A–B) or squirrel monkey (C–D) brainstem. Regions of lower (A,C) and upper (B,D) medulla oblongata are shown. AP—area postrema; ASP—area subpostrema; NTS—nucleus tractus solitarius; TS—tractus solitarius; DMV—dorsal motor nucleus of vagus nerve; XII—hypoglossal nucleus; NG—nucleus gracilis; NC—nucleus cuneatus; LCN—lateral cuneate nucleus; NTST—nucleus of trigeminospinal tract; PCRF—parvicellular retucular formation, including the site of the vomiting center; RFG—nucleus reticularis gigantocellularis; PP—nucleus prepositus; MV—medial vestibular nucleus; LV—lateral vestibular nucleus; IV—inferior vestibular nucleus; SV—superior vestibular nucleus; CRF—corpus restiformis; IO—inferior olive; Py—pyramid. Broken lines encircle structures dissected out as individual tissue blocks for binding assays (bovine brainstem) or corresponding regions of squirrel monkey brainstem (e.g., VNC—vagal nuclear complex; VC—vestibular complex).

ing reticular formation and limbic structures may be important either in the antimotion sickness effects of scopolamine or in the adverse reactions to this drug [16].

Animals used in models of motion sickness generally include cats, dogs or monkeys because common laboratory rodents cannot vomit. One suitable motion sickness model is the squirrel monkey test for motion-induced emesis [7,21]. This model is especially relevant because the aversive motion stimuli (vertical rotation and horizontal acceleration) are the same forces which elicit motion sickness in man and the incidence, latency and frequency of emetic episodes is similar to that observed in humans subjected to analogous test conditions [20,21]. Furthermore, the neural mechanisms involved in motion sickness are alike in the two species [7,21].

It has recently been suggested that neurotransmitter receptor binding assays may be useful in predicting the therapeutic potential of antiemetic drugs and drug combinations [23]. Hence, a relatively simple and inexpensive *in vitro* test could define the pharmacological actions of antiemetics at specific receptors in brain tissue. Biochemical and pharmacological properties of muscarinic cholinergic receptors in bovine brainstem were assessed, in the present study, using ^{3}H-QNB radioligand binding techniques. Such experiments are an essential prerequisite to more detailed autoradiographic studies in brain regions such as area postrema. Bovine brains provided large amounts of tissue for this binding assay and such tissue was readily available from many animals at a minimal cost. However, little is known about emetic mech-

anisms in this species, other than the fact that ruminants, including cattle, can vomit [1,19].

The experiments described in this paper combined radioligand binding assays and neuroanatomical techniques in bovine brainstem with pharmacological studies of motion-induced emesis in squirrel monkeys to investigate motion and space sickness. The hypothesis that antiemetic drugs may act, in part, by blocking brainstem muscarinic receptors was tested. This multidisciplinary approach may further define the biochemical mechanisms and sites of action of antiemetics and antimotion sickness drugs.

METHOD

Tissue Preparation and Dissection

Five brainstem areas were carefully hand dissected from a series of bovine brains:AP; vagal nuclear complex (VNC), including DMV, area subpostrema (ASP) and NTS; parvicellular reticular formation (PCRF), including the site of the vomiting center; nucleus reticularis gigantocellularis (RFG) in the upper half of the medulla oblongata; and (VC), including the medial (MV), lateral (LV), inferior (IV) and superior (SV) vestibular nuclei. These five areas of bovine or squirrel monkey brain are outlined in Fig. 1.

The bovine brains were removed from freshly slaughtered cattle and placed in plastic sacks embedded in crushed ice within 30 min *post mortem*. Dissection of the tissue was done in a cold chamber at −15°C. After dissection (2–6 hours *post mortem*), some of the tissue was stored at −70°C for up to 60 days prior to biochemical analysis. Other dissected tissue blocks were fixed by immersion in a mixture of 10% formalin, glacial acetic acid and methanol (FAM). The fixed tissue was imbedded in paraffin, sectioned serially (10 μm) in the transverse plane and stained with toluidine blue and acid fuchsin. The accuracy of the dissection procedure was verified using these histological preparations. In some brains, the entire pons and medulla were processed similarly for histological study.

The squirrel monkey tissues, used for histological comparisons to the bovine tissues, were obtained from three young adult Bolivian squirrel monkeys. These animals were anesthetized with ketamine (10 mg/kg, IM) and pentobarbital (10 mg/kg, IV) and the brains were fixed by left ventricular cardiac perfusion with isotonic saline for one minute followed without interruption by 10% neutral formalin for twenty minutes. The brainstems were processed for histological study as described for the bovine brains.

Brainstem tissue used in the binding assay was thawed, homogenized (two, 15 sec bursts of a Tekmar Tissumizer at setting = 5) in 40 ml, 0.05 M phosphate buffer (81 mM Na^+, 9 mM K^+; pH=7.4) and centrifuged at 40,000 × g for 10 min (Beckman J2-21). The resulting pellet was resuspended by homogenization in buffer and the process was repeated twice. Muscarinic receptors were assayed directly in this thrice-washed, crude membrane preparation, as described below. Protein content was determined using Bio-Rad protein dye reagent with bovine serum albumin as a standard [5].

Radioligand Binding Assay

Binding of ^{3}H-*l*-quinuclidinyl benzilate (^{3}H-QNB), a potent muscarinic cholinergic antagonist, was assayed in discrete regions of bovine brainstem according to previously described methods [31]. Specific ^{3}H-QNB binding was defined experimentally as the difference between radioactivity bound in the absence and presence of 10 μM atropine. In most cases, specific binding represented 90–96% of total binding. Aliquots of brainstem tissue (0.02–0.20 mg protein), ^{3}H-QNB (20–500 pM for saturation studies, 200 pM for drug inhibition experiments) and various drugs were added to tubes containing a final volume of 2 ml phosphate buffer and the mixture was incubated to equilibrium (60 min at 37°C). The binding reaction was terminated by filtration under reduced pressure through Whatman GF/B glass fibre filters. Each filter was rinsed three times with 4 ml cold buffer using a Brandel model M24 automated filtration apparatus. The radioactivity remaining on the filters was extracted in 5 ml Beckman Ready-Solv EP scintillation cocktail and quantitated in a Beckman LS 7500 liquid scintillation counter (35% efficiency).

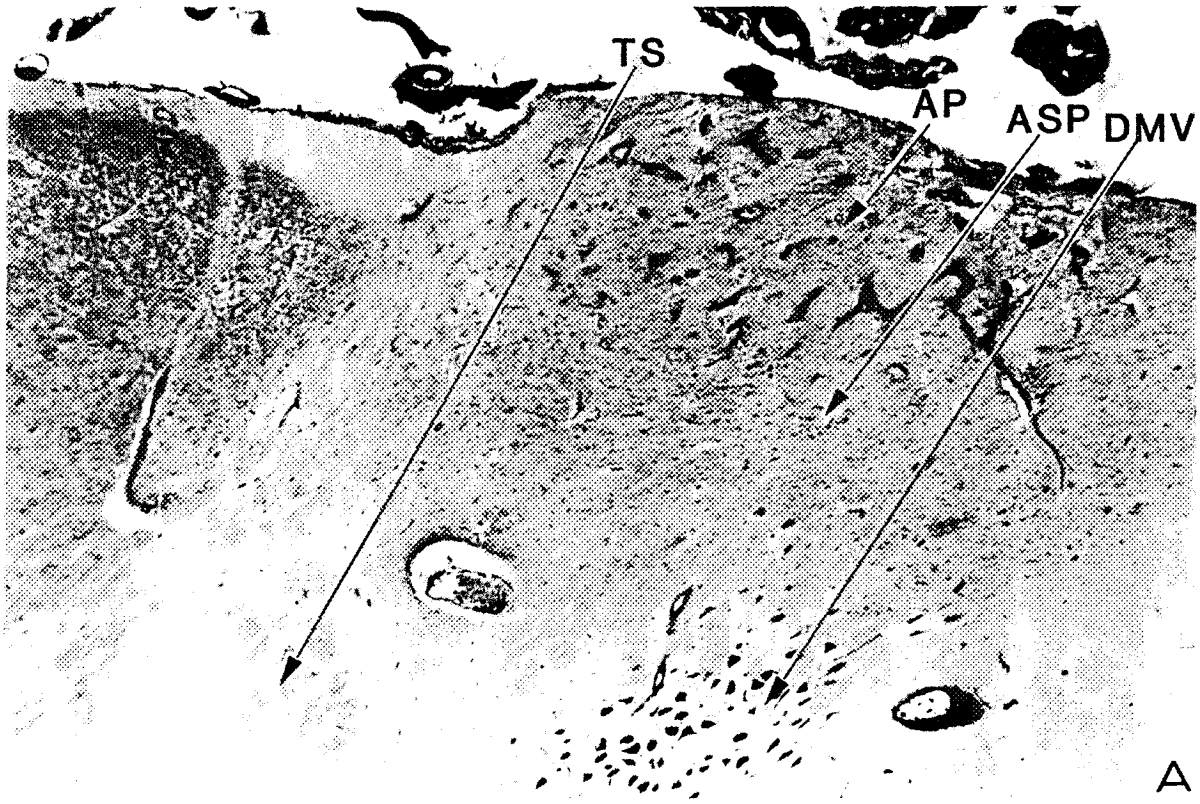
For kinetic experiments, a batch method was used wherein large volumes of incubation media (40–60 ml) were prepared in three separate flasks representing total, nonspecific and displaceable binding. Two ml aliquots of incubation media were removed from the appropriate flask at various times (2–240 minutes after the addition of tissue) and pipeted over individual filters, rinsed and counted as described above. The "total" flask contained ^{3}H-QNB (200 pM) and tissue (2.5 mg wet weight/ml) in a final volume of 60 ml. "Nonspecific" and "displaceable" flasks were prepared similarly, except 10 μM atropine was added initially to the "nonspecific" flask, and at 60 min to the "displaceable" flask. The time course of association and dissociation of specific ^{3}H-QNB binding could thus be determined, as previously described [10].

Tests of Antiemetic Activity

The antiemetic efficacy of various drugs was evaluated in young adult squirrel monkeys (Bolivian subspecies) weighing from 500–700 g, which have been shown to be excellent subjects for motion-sickness studies [7,21]. Motion-induced emesis was elicited in each monkey via a combination of continuous counterclockwise horizontal rotary motion (25 rpm) and a sinusoidal vertical excursion of 15 cm every 2 sec for a period of one hour, as described previously [7]. Criterion for emesis was forcible expulsion of stomach contents through the mouth, and the incidence, frequency and latency of emetic episodes were recorded. Each monkey was given 30 g of banana, 10–15 min prior to the emetic test, to standardize food intake.

Monkeys were exposed to the motion stimulus only once a week, with a six day rest period between tests, in order to minimize habituation to motion-induced emesis. Placebo and drug injections were alternated over a seven week period, each animal receiving four placebo and three drug exposures. Doses of antiemetic drugs, selected from pilot studies or extrapolated from effective human dose levels, were as follows: chlorpromazine, 0.36 mg/kg; domperidone, 6.0 mg/kg; fluphenazine, 0.36 mg/kg; prochlorperazine, 0.25 mg/kg; thiethylperazine, 0.30 mg/kg. All drugs were given intramuscularly 45 min prior to the motion regimen.

Only monkeys who maintained a frequency of 2–5 emetic episodes for each placebo test were counted in this study. Each drug was tested in two or three monkeys and the average number of emetic episodes per one hour session was determined. An antiemetic score for each drug was calculated as (P-D/P × 100), where P is the average number of emetic episodes after placebo and D is the average number of emetic episodes after a single dose of antiemetic drug. Thus, a score of 100% represented a totally effective antiemetic,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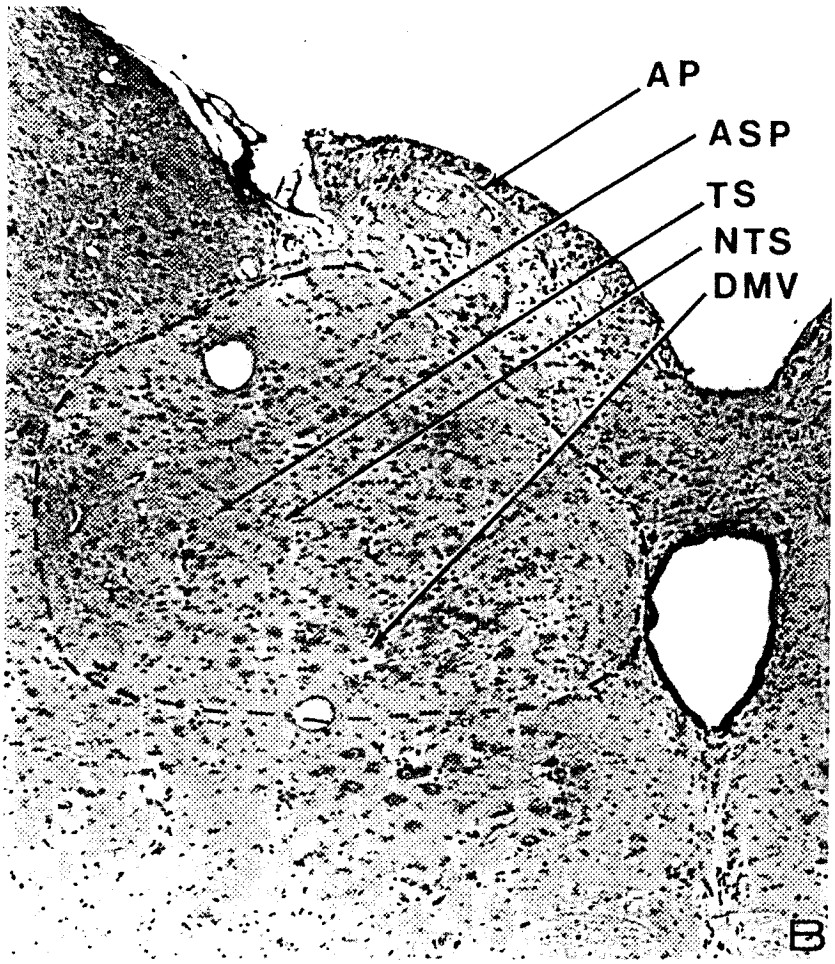
AP
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DMV
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whereas a score of 0% indicated a lack of antiemetic activity, and each animal served as its own control.

Drugs and Chemicals

Quinuclidinyl benzilate-*l*-[benzillic-4, 4′-^{3}H] (^{3}H-QNB) was purchased from New England Nuclear (Boston, MA) at a specific activity of 26.8 Ci/mmol. Atropine sulfate, chlorpromazine hydrochloride and diphenhydramine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Prochlorperazine and thiethylperazine were obtained from Smith, Kline and French Labs (Philadelphia, PA) and Sandoz Inc. (East Hanover, NJ), respectively. Fluphenazine dihydrochloride was the gift of E. R. Squibb and Sons, Inc. (Princeton, NJ).

RESULTS

Histological Comparison of Bovine and Squirrel Monkey Brainstem

The general cross-sectional configuration and locations of pertinent nuclei in squirrel monkey and bovine brainstems are illustrated in Fig. 1 (A–D). The dashed lines in Fig. 1 (A and B) circumscribe bovine brainstem areas dissected for use in radioligand binding studies. Corresponding structures in squirrel monkey brainstem are also encircled by dashed lines (Fig. 1, C and D). The figures are drawn to different scales so as to represent bovine and squirrel monkey tissue in approximately equal dimensions. In fact, bovine brainstem was 5–6 times larger than squirrel monkey brainstem. Figure 2 shows photomicrographs of transversely sectioned bovine (A) and squirrel monkey (B) brainstem at the level of AP and DMV, stained with toluidine blue and acid fuchsin.

Although a detailed comparison of neuronal structures in bovine versus squirrel monkey brainstem is beyond the scope of this paper, certain histological similarities were noted between the two species. In both species, the DMV was located in the median portion of the trigonum vagi and was composed mainly of small spindle-shaped cells, but with some larger cells having coarser chromophillic bodies. The NTS was divisible into a medial part, dorsolateral to the DMV, and a lateral part located along the lateral border of the tractus solitarius (TS). The medial part consisted of small, densely packed cells and the lateral part of slightly larger cells. ASP was actually part of the medial portion of the NTS containing small neurons and merged into the main body of the NTS without any distinct boundary.

Bovine AP was situated somewhat more deeply in the tissues of the dorsal part of the caudal medulla than it was in the squirrel monkey. Except for this difference the AP tissues had essentially the same cell structure and topographic relationships in the two species. It consisted of small neurons, some having Nissl material, and a large number of glial cells. The AP of each species was highly vascular due to the presence of many sinusoidal capillaries.

The reticular formation at the site of the vomiting center (PCRF) was composed almost entirely of small neurons in both species. No particular distinguishing characteristics were observed in toluidine blue-acid fuchsin stained tissues. The RFG of both bovine and squirrel monkey consisted of many large neurons, with a few medium and small cells.

In both species, the VC included the medial, lateral, inferior and superior vestibular nuclei. The MV was situated in the floor of the fourth ventricle medial to the IV and LV and immediately lateral to the nucleus prepositus. The cells were small and medium sized, rather evenly distributed and closely packed. A few larger cells could be seen dorsolaterally in the MV in both species. The LV was located laterally in the ventricular floor at the level of the vestibular nerve. This nucleus was characterized by the presence of giant cells with coarse Nissl granules which appeared to be more numerous in the caudal than in the middle or rostral parts of the nucleus. The IV was composed of small and medium-sized cells, except rostrally where larger cells are found. The SV was dorsal and rostral to the LV. The cells of this nucleus in both species were medium and small, round or spindle shaped and rather loosely scattered. Clusters of larger cells were also observed in this nucleus.

Distribution and Kinetics of ^{3}H-QNB Binding in Bovine Brainstem

Muscarinic cholinergic receptors labeled by ^{3}H-QNB were unevenly distributed in bovine brainstem. Saturation isotherms of specific ^{3}H-QNB binding showed highest receptor densities in the VNC and AP, with intermediate binding levels in RF (PCRF and RFG) and lowest levels in VC (Table 1). The Bmax values for AP expressed as fmol/mg protein were rather high, probably due to the jelly-like consistency of AP and, hence, its relatively low amount of protein per mg wet weight of tissue. Scatchard analyses [24] of these data yielded monophasic plots (Fig. 3), indicative of ^{3}H-QNB binding to a single population of sites over the range of ligand concentrations used (20–500 pM). The apparent dissociation constant (Kd) for ^{3}H-QNB was similar in each of the four areas studied, ranging from 25–56 pM (Table 1).

The time course of specific ^{3}H-QNB binding was also determined in three brainstem areas (VNC, RF and VC). Figure 4 shows a typical time course experiment for bovine VNC. In each area, binding equilibrium was reached at 60 min incubation (at 37°C) and the half-time of dissociation was from 40–60 min. The kinetic Kd's, defined in these experiments as the ratio of the dissociation rate constant (K−1) to the association rate constant (K+1), were similar in all brainstem areas tested (Table 2) and agreed well with the Kd's determined in saturation studies.

Pharmacology of ^{3}H-QNB Binding Sites

Various drugs inhibited specific ^{3}H-QNB binding in bovine VNC, RF and VC (Table 3). Muscarinic antagonists (*dl*-QNB, atropine and scopolamine) were more potent inhibitors than agonist drugs (oxotremorine and arecoline). In addition, drug-inhibition curves for antagonists had Hill slopes close to unity (0.8–1.1), whereas Hill slopes for agonists were more shallow (0.4–0.7) (data not shown). The

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FIG. 2. Photomicrographs showing histological characteristics of area postrema and adjacent structures in bovine (A) or squirrel monkey (B) brainstem (approximate magnification 40× and 64×, respectively). Note the relatively deep location of the bovine area postrema as compared with its superficial location in the squirrel monkey brainstem. AP—area postrema; ASP—area subpostrema; DMV—dorsal motor nucleus of vagus nerve; TS—tractus solitarius.

TABLE 1

SATURATION ISOTHERMS OF ^{3}H-QNB BINDING IN BRAINSTEM AREAS REGULATING EMESIS: SCATCHARD ANALYSES

Brainstem Area	Kd (pM)	Bmax (fmol/mg tissue)	Bmax (fmol/mg protein)
Vagal Nuclear Complex	35.83 ± 4.26	19.96 ± 1.38	1048 ± 139
Reticular Formation-PCRF	35.63 ± 3.20	14.85 ± 1.39	719.2 ± 67.0
Reticular Formation-RFG	36.10 ± 3.98	13.20 ± 1.64	646.8 ± 81.5
Vestibular Complex	25.29 ± 2.53	7.53 ± 0.64	393.9 ± 26.7
Area Postrema	55.66 ± 8.89	10.09 ± 1.71	1023 ± 243

*Data expressed as mean ± SEM for N=3 or 4 separate experiments.

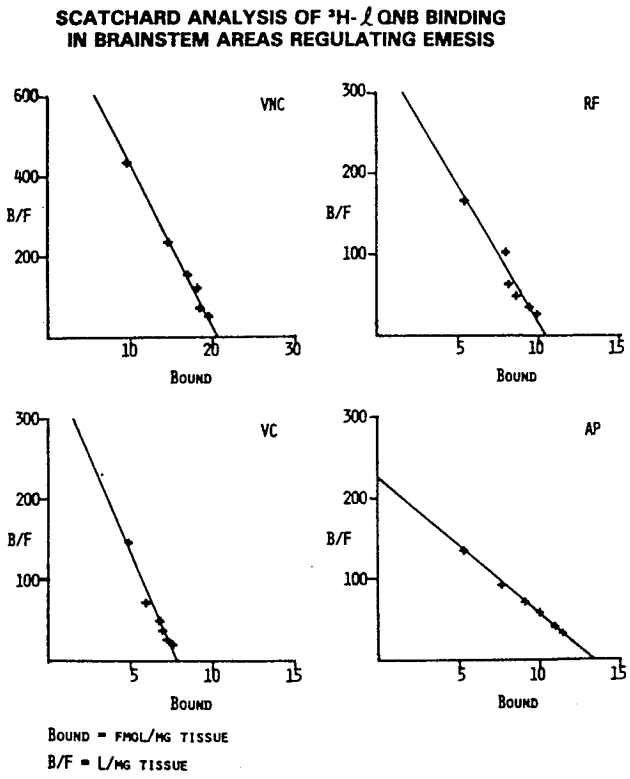

FIG. 3. Representative Scatchard plots of ^{3}H-QNB binding in bovine vagal nuclear complex (VNC), brainstem reticular formation (RF), vestibular complex (VC) and area postrema (AP).

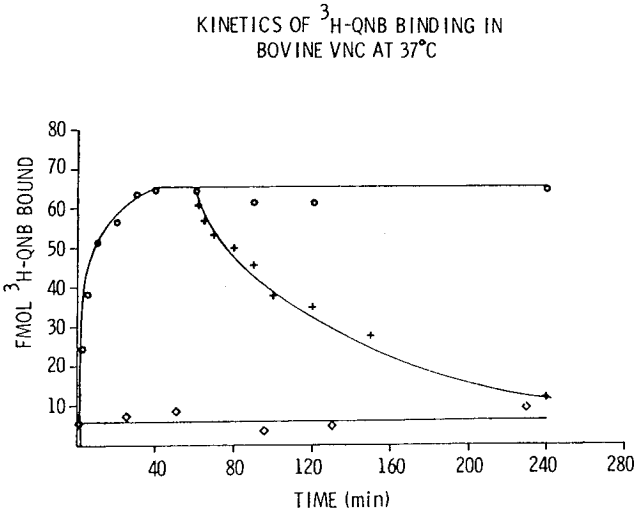

FIG. 4. Time course of ^{3}H-QNB binding in bovine vagal nuclear complex. Tissue was added at time 0 to flasks containing ^{3}H-QNB (200 pM) and buffer, with (◇) or without (○) 10 μM atropine. At equilibrium (60 min), 10 μM atropine was added (+) to displace bound ^{3}H-QNB. Similar results were obtained in bovine vestibular complex and reticular formation (see Table 2).

rank order of potency for cholinergic drugs was similar in each brainstem area, i.e., *dl*-QNB > atropine = scopolamine >> oxotremorine > arecoline.

Antiemetic drugs inhibited brainstem ^{3}H-QNB binding with Ki values in the nanomolar to micromolar range (Table 3). The phenothiazine drugs promethazine, chlorpromazine and prochlorperazine, as well as the antihistamine diphenhydramine, were the most potent muscarinic antagonists tested. Thiethylperazine, fluphenazine and domperidone were successively weaker inhibitors of ^{3}H-QNB binding. Again, there were no apparent differences between the three brainstem areas in the rank order of potency of these drugs.

Antiemetic Efficacy of Drugs in the Squirrel Monkey Motion Sickness Test

Of the five drugs tested for their ability to inhibit motion-induced emesis in squirrel monkeys, chlorpromazine and prochlorperazine were most effective. These drugs completely prevented vomiting in each of nine drug tests in three different monkeys. Thiethylperazine and fluphenazine were less effective antiemetics, whereas domperidone had relatively little antiemetic activity in this procedure. Table 4 summarizes these results.

TABLE 2

TIME COURSE OF ^{3}H-QNB BINDING AT 37°C

Brainstem Area	Kinetic Kd (pM)	Dissociation Rate (min^{-1})	Association Rate ($pM^{-1}\ min^{-1} \times 10^3$)
Vagal Nuclear Complex	27.62	0.0116	0.420
Reticular Formation	31.50	0.0172	0.546
Vestibular Complex	24.26	0.0115	0.474

TABLE 3

DRUG INHIBITION OF ^{3}H-/QNB BINDING IN BRAINSTEM AREAS REGULATING EMESIS

		Vagal Nuclear Complex		Reticular Formation		Vestibular Complex	
Class	Drug (n)	IC50	Ki	IC50	Ki	IC50	Ki
Cholinergic Antagonists	d/-QNB (3)	0.471± 0.097	0.089	0.742± 0.276	0.118	0.497± 0.099	0.058
	Atropine (3)	16.7 ± 2.57	3.72	14.7 ± 0.16	2.72	14.2 ± 3.81	1.83
	Scopolamine (3)	12.9 ± 1.32	2.47	29.9 ± 11.1	5.22	15.6 ± 3.75	1.95
Cholinergic Agonists	Oxotremorine (4)	1410 ± 498	267	2330 ± 501	355	1450 ± 319	164
	Arecoline (4)	14,500 ±2490	2750	28,600 ±8540	3500	29,600 ±4070	3610
Phenothiazines	Promethazine (3)	73.2 ± 10.9	14.0	102 ± 46.6	18.8	102 ± 16.3	12.5
	Chlorpromazine (3)	899 ± 172	171	960 ± 66.4	149	993 ± 83.8	128
	Prochlorperazine (3)	3690 ± 694	714	4580 ±1850	823	5450 ±1240	708
	Thiethylperazine (3)	8210 ± 403	1810	14,200 ±3190	2560	9450 ± 905	1230
	Fluphenazine (3)	13,500 ±5830	2740	35,100 ±6980	2620	14,000 ±2330	1750
Miscellaneous	Diphenhydramine (3)	1250 ± 134	254	800 ± 159	144	1160 ± 347	137
	Domperidone (3)	27,000 ±2130	4660	33,500 ±2530	4940	28,500 ±1710	3080

IC50's are expressed in nM ± SEM for N=3 or 4 separate experiments; Ki's are calculated as $IC50/1+\frac{[^3H\text{-}/QNB]}{K_d}$, where K_d=43.55, 36.10, and 25.29 pM in VNC (vagal nuclear complex), RF (reticular formation) and VC (vestibular complex), respectively.

DISCUSSION

The major findings of these studies were (1) the cellular organization of brainstem nuclei was histologically similar in bovine and squirrel monkey brain; (2) some, but not all drugs which prevented motion-induced emesis in squirrel monkeys also inhibited ^{3}H-QNB binding in bovine brainstem; (3) pharmacological properties of the muscarinic receptors in brainstem areas regulating emesis were identical to previously reported characteristics of ^{3}H-QNB binding in other brain regions and in peripheral tissues; (4) there was an uneven distribution of ^{3}H-QNB binding sites in bovine brainstem, including the unique observation of muscarinic receptors in AP.

Since the biochemical assays and antiemetic tests were done in two different species, it was important to demonstrate that brainstem structures regulating emesis were histologically similar in squirrel monkey and bovine brains. Such a structural and topographical similarity was confirmed in the present study using toluidine blue-acid fuchsin staining techniques.

TABLE 4

ACTIVITY OF VARIOUS DRUGS IN PREVENTING MOTION-INDUCED EMESIS IN SQUIRREL MONKEYS

	Placebo*	Drug*	Antiemetic Activity†
Chlorpromazine (0.36 mg/kg, IM)	3.83 ± 0.30	0.00	100%
Prochlorperazine (0.25 mg/kg, IM)	3.17 ± 0.27	0.00	100%
Thiethylperazine (0.30 mg/kg, IM)	3.13 ± 0.44	0.83 ± 0.54	73%
Fluphenazine (0.36 mg/kg, IM)	3.25 ± 0.31	1.17 ± 0.40	64%
Domperidone (6.0 mg/kg, IM)	4.86 ± 0.14	3.00 ± 0.77	38%

*Average number of emetic episodes per one hour session ± SEM (see the Method section).

†Calculated as (Placebo-Drug/Placebo × 100).

This is apparently the first report describing the neuronal histology of bovine brainstem. The most notable difference between bovine and squirrel monkey brainstem was the raised contour of squirrel monkey AP compared to the more deeply situated bovine AP. Otherwise the arrangement of AP, NTS, DMV, VC and adjacent areas was remarkably similar in the two species. Comparison of cells within these structures after Nissl staining showed that, in both species, DMV consisted mainly of small, spindle-shaped cells; NTS of small, densely packed neurons; AP of small neurons, with some Nissl material, and a large number of glia; PCRF of small neurons, entirely; and RFG of many large neurons, with a few smaller cells. Cellular structures within the different vestibular nuclei (MV, LV, IV and SV) showed no marked species differences.

Emetic mechanisms have not been thoroughly studied in ruminant animals. However, there is at least one report of vomiting in cattle and sheep, which occurred after the administration of veratrum alkaloids [19]. The authors concluded that ruminants can exhibit emesis, apparently mediated by central systems and similar to that observed in animals with a single stomach. It is further suggested that ruminants may have a brainstem vomiting center similar to that found in dog or man. In support of that possibility, electrical stimulation of brainstem areas lateral to AP has been shown to induce vomiting in conscious goats [1]. However, stimulation of PCRF in goats causes retching, but not vomiting. Results of the present study confirmed the similarities between ruminant and primate brainstem areas regulating emesis as defined by histological techniques.

Brainstem tissue from approximately 200 bovine brains was required to complete the binding assays described in this study. Comparable studies in rats, dogs or squirrel monkeys would necessitate the sacrifice of 500–1000 animals to obtain sufficient amounts of brainstem tissue. Whereas bovine brains were easily obtainable, other sources of tissue would be prohibitively expensive.

A theory of the mechanism of action of antimotion sickness drugs has been proposed based upon the pharmacological activity of such agents [30]. These authors stress the importance of antimuscarinic and sympathomimimetic activity in preventing motion-induced emesis. It is generally agreed that the single most effective antimotion sickness drug is the muscarinic antagonist scopolamine [6, 29, 30]. Furthermore, recent work has documented a physiological basis for the role of central cholinergic systems in modulating the perception of motion stimuli and expression of motion sickness symptoms [16].

The results shown in Tables 3 and 4 indicate a possible association between antiemetic efficacy and inhibitory potency at ^{3}H-QNB binding sites for the five drugs tested. Unambiguous conclusions cannot be made because quantitative data showing dose-dependent drug effects in the squirrel monkey model are lacking. However, it is likely that the inhibition of motion-induced emesis by drugs like chlorpromazine and prochlorperazine are due, in part, to their antimuscarinic activities. In contrast, domperidone, fluphenazine and thiethylperazine were weaker inhibitors of ^{3}H-QNB binding than chlorpromazine or prochlorperazine and were only partially effective at preventing motion-induced emesis.

The biochemical characteristics of ^{3}H-QNB binding in bovine brainstem were similar to those observed in other brain regions and peripheral tissues. Although the time course of ^{3}H-QNB binding in brainstem tissues has not been described previously, the kinetic constants reported here are similar to values obtained in rat heart [10]. Saturation studies revealed and kinetic analyses confirmed the high affinity binding of ^{3}H-QNB in brainstem areas, with Kd values of 20–60 pM. These results agreed well with reports of ^{3}H-QNB binding in rat ileum, forebrain, brainstem, cerebellum and heart [9,10]. In addition, the rank order of potency of cholinergic agonists and antagonists at inhibiting ^{3}H-QNB binding was the same in brainstem regions as in other tissues [9, 10, 31]; a pharmacological profile consistent with binding to muscarinic cholinergic receptors. Although there were significantly different densities of muscarinic receptors in the various brainstem areas regulating emesis, regional differences in the pharmacological properties of ^{3}H-QNB binding were not observed in the present study.

The initial reports of ^{3}H-QNB binding in brainstem [12, 15, 25] have been substantially extended by the present investigation, as the cholinergic pharmacology of these sites was confirmed and a better estimate of receptor density and kinetics was provided. The high density of muscarinic receptors in VNC, with lower levels of ^{3}H-QNB binding in RF and VC is generally consistent with earlier reports [12, 15, 25, 28]. The regional distribution of choline acetyltransferase, high affinity choline uptake and acetylcholinesterase [14,25] in rat brainstem also parallels the distribution of ^{3}H-QNB binding sites in bovine tissue.

Although muscarinic receptors in rat brainstem have been identified previously using ^{3}H-QNB binding [12, 15, 25] or autoradiographic techniques [28], this is the first report showing muscarinic receptors in AP. The AP is a crucial region mediating chemically-induced emesis [2], but its influence on nausea and vomiting associated with motion-sickness is unclear [7,19]. Muscarinic receptors in AP could offer a pharmacological target for anticholinergic drugs which do not cross the blood-brain barrier. If antagonism of cholinergic systems in AP directly inhibited emesis, then peripherally-acting antimuscarinics, such as methscopolamine, could prove useful in treating symptoms of motion sickness. Use of methscopolamine in motion sickness would likely minimize central side-effects of scopolamine therapy, such as sedation and, possibly, loss of visual accomodation [16]. Similar reasoning suggests the possible utility of peripheral anticholinergics in alleviating drug-induced nausea and vomiting, which can be especially debilitating in cancer patients receiving chemotherapy [22]. These potential therapeutic applications warrant further investigation.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Lerna Minor, Mary McDonald and Mel Weaver for their expert technical assistance, and Wanda M. Cordova and Thelma A. Evans for their help in preparing this manuscript. This work was supported in part by the E. G. Schlieder foundation, NASA-Ames NAC-2-101 and NIH RR00164.

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